Title of the thesis: ***Understanding the translation regulation of Fragile X Mental Retardation Protein (FMRP) through its interaction with the ribosome***

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Introduction: (Chapter 1 of the thesis)

Fragile X Syndrome (FXS) is a Trinucleotide repeats expansion disorder of the FMR1 gene resulting in intellectual disability [1]. The silencing of the gene due to the expansion of CGG repeat in its 5'UTR results in the loss of the encoded protein Fragile X Mental Retardation Protein (FMRP). FMRP is an RNA binding protein, structurally comprising of protein interacting Tudor and KH0 domains and two types of RNA binding domains: two K homology domains and one RGG domain in the intrinsically disordered C-terminus [2–5]. FMRP is implicated in a range of biological processes such as chromatin remodeling, ion channel stability, RNA transport, ribosome heterogeneity, and translation regulation[6–12]. However, the contribution of FMRP to FXS pathology is mostly studied in the absence of the protein and the role of the individual domains of FMRP in these cellular processes is yet to be investigated.

FMRP plays a key role in maintaining synaptic development and plasticity through regulation of protein synthesis[13,14]. FMRP is primarily shown to act as a translational repressor[15]. However, FMRP also activates the translation of specific candidates both on mGluR and NMDAR stimulation[10,11]. It is important to note that FMRP bound targets localize to neuronal processes in the form of granules in a microtubule-dependent manner and the expression of these targets is guided through FMRP's distribution to polysomes[16–19]. The interaction of FMRP with ribosomes seems to be an important prerequisite for this. An added level of translation regulation lies in the phosphorylation of FMRP. Phosphorylation of FMRP, primarily at Serine-500, is shown to influence the bidirectional role of FMRP as a regulator of synaptic protein synthesis[20–23].

The function of FMRP comprises the multistep process of granule formation, association with microtubules and interaction with ribosomes. Only a few studies address the mechanism of these interactions through truncations and mutations of FMRP. The N-terminus domain of FMRP is shown to modulate the expression and activity of several ion channels while the RGG domain in the C-terminus is implicated in RNA binding and consequent gene expression[24–27]. Further, the I304N mutation of FMRP, which results in a severe form of FXS, illustrates the importance of KH domains in polysome association and translation regulation [18,28]. Apart from I304N, gene sequencing studies have uncovered a series of other mutations in FMR1 leading to FXS-like phenotypes. In-silico analysis of FMR1 SNPs indicate that R138Q mutation in the NLS of FMRP alters the localization of FMRP without any structural effect on the protein[2,29]. But, mutations in the KH domains like G266E and I304N seem to disturb the interactions between domains, disrupting the overall structure and function of FMRP[29,30]. C-terminus domain mutations such as

R534H and G482S have been predicted to alter RNA binding and recognition [31]. In addition, the frame shift mutation (G538fs*23) in the open reading frame of FMRP has been shown to introduce novel sequences, which drastically alter the cellular localization of FMRP[32]. These mutational studies emphasize the probable structural defects and the contribution of individual domains of FMRP. However, the consequence of these point mutations in cellular processes is yet to be addressed. Further, this has generated a vacuum in the information available to understand the functions manifested by FMRP mutants.

As an RBP, FMRP is known to localize to the cytoplasm as well as the nucleus[33–35]. A significant amount of FMRP is also known to localize to the nucleolus in various cell types[36]. However the role of FMRP in these cellular compartments is yet to be elucidated. Since FMRP has both Nuclear Localization Signal (NLS) and Nucleolar Export Signals (NES), it has been proposed to regulate the shuttling of mRNAs between the nucleus and the cytoplasm[35]. Additionally nuclear FMRP has also been implicated in chromatin remodeling[6]. A mutation in this nuclear localization signal was also reported to cause Fragile X-like syndrome[37]. Further, there is an intriguing possibility that FMRP might interact with a distinct class of RNA in the nuclear/nucleolar compartments and play a crucial role in global translation regulation. This suggests that FMRP has an important function in the nucleus, which could modulate its function in the cytoplasm.

The function of FMRP is complex and studying the localization and role of individual domains is beneficial in understanding FMRP-mediated regulation of protein synthesis. Hence we systematically investigated the contribution of FMRP and its domains in the dynamic processes of ribosome binding, mRNP granule formation, and microtubule association. We also emphasize the role of phosphorylation of FMRP at Serine 500 in these processes. Additionally, we elucidate the functional consequences of pathogenic point mutations on regulatory mechanisms of FMRP. Lastly, our study also describes a novel role for nuclear FMRP and how its interaction with nuclear small RNA can regulate the ribosome and thus protein synthesis. This is important to understand FMRP domains and their role in coordinating the mechanisms leading to translation regulation.

Hypothesis: Distinct domains of FMRP regulate protein synthesis through their interaction with the ribosome.

Objectives:

1. To investigate the regulation of translation by FMRP domains through their interaction with the ribosome.
2. To investigate the contribution of FMRP domains in regulating puncta formation and microtubule association
3. To validate the role of FMRP domains in translation regulation through the use of pathogenic FMRP mutations
4. To examine the role of nuclear FMRP in regulating protein synthesis

Model system:

Recombinant proteins corresponding to full-length FMRP and individual domains were purified from E.coli and Sf9 insect cells. These were used in in vitro binding assays. Rat primary cortical neurons and HEK293T cells were used for all over-expression assays.

Materials and methods:

Materials and methods used in this study are publish in **'***Function of FMRP Domains in Regulating Distinct Roles of Neuronal Protein Synthesis*', Molecular neurobiology **2022 DOI: 10.1007/s12035-022-03049-1** and in '*FMRP Interacts with C/D Box snoRNA in the Nucleus and Regulates Ribosomal RNA Methylation'*. **iScience 2019 DOI: 10.1016/j.isci.2018.11.007**

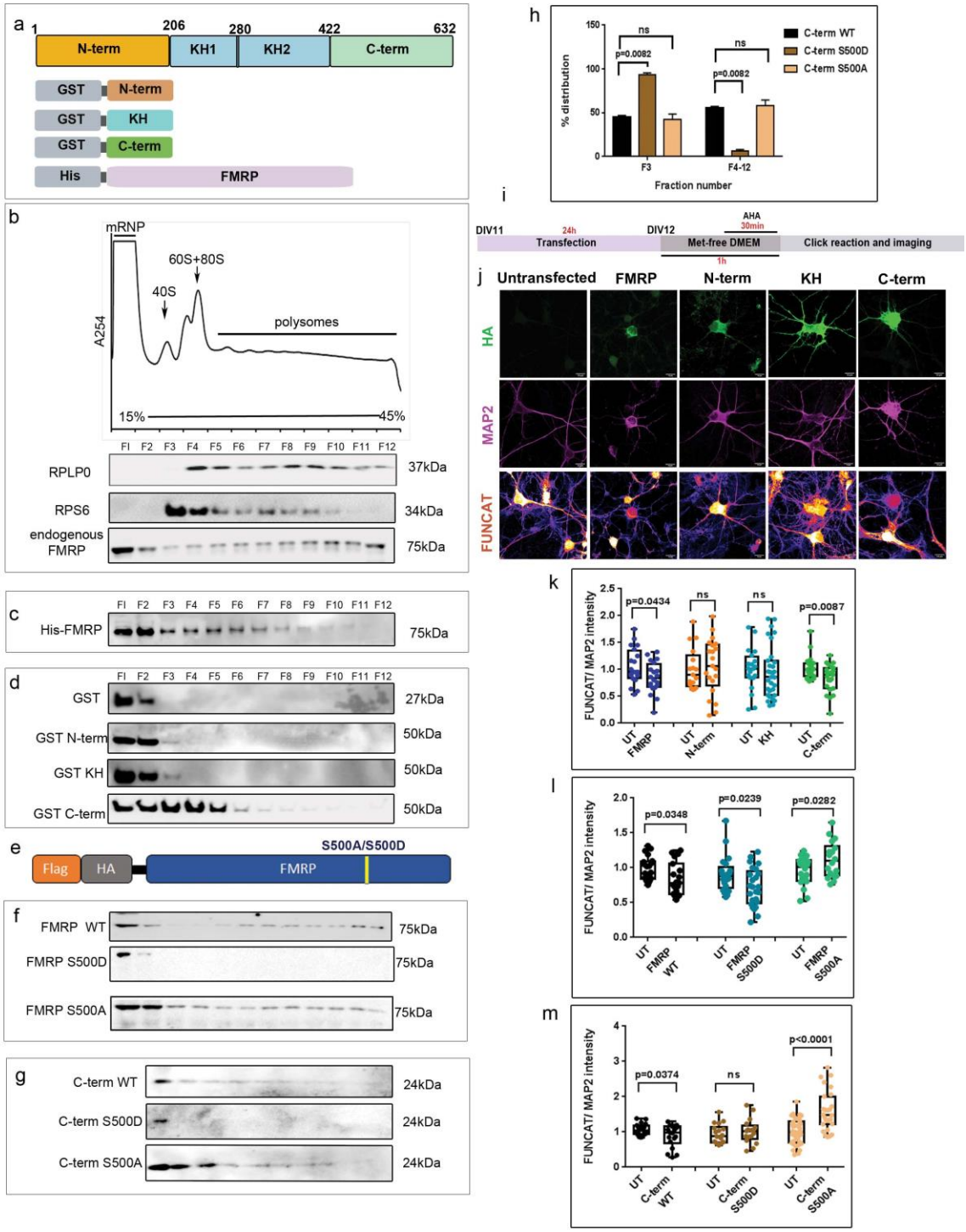
Results:

1. **Regulation of translation by FMRP through its interaction with the ribosome.**

FMRP is a modulator of activity-mediated translation in the nervous system [14,38,39]and one of the requisites for this function is the ability of FMRP to interact with ribosomes and polysomes[40]. This feature was clearly shown in I304N FMRP mutant, which does not associate with polysomes, but retains its RNA binding capacity[18]. FMRP is a multi-domain protein and it is important to understand which of these domains is essential for its interaction with ribosomes. We fragmented human FMRP into three parts, the N-terminus (N-term), the KH1 and KH2 domains (collectively called the KH domain here) and the C-terminus containing the RGG box (C-term). Recombinant proteins corresponding to the individual domains and full-length FMRP were spiked into HEK293T cell lysate subjected to polysome profiling to test their association with ribosomes and polysomes. From our results, FMRP associated with all fractions of the gradient including polysomes. Interestingly, only the C-terminus alone was capable of associating with ribosomes and polysomes.

Phosphorylation of FMRP is known to modulate majority of its functions and the primary phosphorylation site of FMRP is Serine500, which is located in its C-terminus domain[20–22,41]. We next investigated if phosphorylation could modulate the extent of ribosome/ polysome association. We over-expressed WT, phosphomimetic (S500D) and de-phosphomimetic (S500A) variants of FMRP in HEK293T cells and observed that dephosphorylation increased its association with polysomes while phosphorylation resulted in accumulation among the initial fractions. We obtained similar results with WT, S500D and S500A forms of C-terminus alone. Dephosphorylation of Cterm increased its association with polysomes whereas phosphorylation increased its accumulation in the initial repressive fractions of the gradient.

We next tested the contribution of individual domains on protein synthesis repression in neurons. Rat primary cortical neurons were transfected with full length and individual domains of FMRP and changes in de novo protein synthesis we assayed through FUNCAT. We observed an overall decrease in FUNCAT signal in neurons transfected with full length FMRP and C-term domain alone in comparison to untransfected neurons. Interestingly dephosphorylation of both full length FMRP and C-term at Serine 500 resulted in an increase in the FUNCAT signal and corresponding increase in protein synthesis. Thus confirming that C-term alone is necessary and sufficient to drive FMRP mediated translation repression in neurons and phosphorylation at Serine 500 can modulate this function.


a
1
206
280
422
632
N-term
KH1
KH2
C-term
GST
N-term
GST
KH
GST
C-term
His
FMRP
b
mRNP
60S+80S
40S
polysomes
A254
15%
45%
Fl F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
RPLP0
37kDa
RPS6
34kDa
endogenous FMRP
75kDa
c
Fl F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
His-FMRP
75kDa
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GST
27kDa
GST N-term
50kDa
GST KH
50kDa
GST C-term
50kDa
e
S500A/S500D
Flag
HA
FMRP
f
FMRP WT
75kDa
FMRP S500D
75kDa
FMRP S500A
75kDa
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C-term WT
24kDa
C-term S500D
24kDa
C-term S500A
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100
50
0
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p=0.0082
ns
p=0.0082
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F4-12
Fraction number
C-term WT
C-term S500D
C-term S500A
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Transfection
DIV12
AHA
30min
Met-free DMEM
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FMRP
N-term
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C-term
HA
MAP2
FUNCAT
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FUNCAT/ MAP2 intensity
2.5
2.0
1.5
1.0
0.5
0.0
p=0.0434
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ns
p=0.0087
UT
FMRP
UT
N-term
UT
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FUNCAT/ MAP2 intensity
2.0
1.5
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p=0.0348
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p=0.0282
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FMRP WT
UT
FMRP S500D
UT
FMRP S500A
m
FUNCAT/ MAP2 intensity
4
3
2
1
0
p=0.0374
ns
p<0.0001
UT
C-term WT
UT
C-term S500D
UT
C-term S500A

Figure 1: C-terminus of FMRP is essential and sufficient for ribosome binding and translation repression.

a. *Schematic of the constructs for individual domains and full-length human FMRP. The GST tagged domains and His-tagged full-length FMRP were expressed in bacteria and Sf9 insect cells respectively. Purified proteins were spiked into HEK293T cell lysate and subjected to polysome profiling on a linear sucrose gradient.*
b. *Top - Representative polysome trace of HEK293T cell lysate on 15%-45% linear sucrose gradient. Bottom – Representative immunoblots for endogenous FMRP, RPLP0 and RPS6 distribution in the 12 fractions obtained from polysome profiling. (n=3).*
c. *Representative immunoblot for spiked His-tagged full-length FMRP distribution in the 12 fractions obtained from polysome profiling probed with anti-His antibody (n=3).*
d. *Representative immunoblots indicating the distribution of spiked GST-tagged FMRP domains and purified GST in the 12 fractions obtained from polysome profiling. Blots were probed with anti-GST antibody. (n=3)*
e. *Schematic describing WT FMRP, S500D and S500A mutants of FMRP that were transfected in HEK293T cells for 24h before subjecting it to polysome profiling on a linear sucrose gradient.*
f. *Representative immunoblots indicating the distribution of overexpressed full-length WT FMRP, phosphomimetic S500D FMRP and dephospho-mimetic S500A FMRP along the 12 fractions of linear sucrose gradient.*
g. *Representative immunoblots indicating the distribution of overexpressed C-term WT, phospho-mimetic (C-term S500D) and dephospho-mimetic (Cterm- S500A) along fractions 3 to 12 of a linear sucrose gradient. Blots were probed with anti-HA antibody (n=3).*
h. *Graph indicating the quantification of overexpressed C-term variants in ribosomal subunit fraction (F3) versus Ribosomal fractions (F4-12). n=3 for each condition. Data represented as mean +/- SEM, One-Way ANOVA p=0.004 for F3, p=0.004 for F4-12 followed by Bonferroni's Multiple comparisons test.*
i. *Experimental workflow. Primary rat cortical neurons (DIV11) were transfected for 24h with full-length and FMRP domains. Transfected neurons were subjected to fluorescent non-canonical amino acid tagging (FUNCAT) along with immunostaining for HA and MAP2.*
j. *Representative images for HA, MAP2 and FUNCAT fluorescent signal in neurons transfected with full-length FMRP and FMRP domains (Scale bar - 10µm)*
k. *Quantification of the FUNCAT fluorescent intensity normalized to MAP2 fluorescent intensity for full-length and domains of FMRP. For each FMRP construct, the FUNCAT signal from the transfected neuron was normalized to the untransfected neuron. Whiskers range from minimum to maximum data point. Unpaired t-test for each construct, n = 15-30 neurons from 5 independent experiments*
l. *Quantification of the FUNCAT fluorescent intensity normalized to MAP2 fluorescent intensity for full-length WT, S500D and S500A FMRP. Unpaired t-test for each construct, n = 20-40 neurons from 4 independent experiments*
m. *Quantification of the FUNCAT fluorescent intensity normalized to MAP2 fluorescent intensity for C-terminus WT, S500D and S500A variants. Unpaired t-test for each construct, n = 20-40 neurons from 4 independent experiments.*

2.Contribution of FMRP domains in regulating puncta formation and microtubule association

FMRP target mRNA localize to neuronal processes in the form of granules/ puncta in a microtubule dependent manner[16,40]. To gain more insights on the contribution of FMRP's domains to puncta formation, we transfected rat primary cortical neurons with full length and domains of FMRP and measured the size and number of puncta containing the over-expressed proteins. Interestingly none of the individual domains were capable for forming well-defined puncta in comparison to those containing full length FMRP. This indicates that the synergistic contribution of 2 or more domains of FMRP is necessary to form functional neuronal puncta.

On altering the phosphorylation status of FMRP at serine 500, we observed that dephosphorylation increased the overall size and number of puncta. Conversely phosphorylation of FMRP caused a reduction in the size and number of neuronal puncta. We next investigated the translational status of FMRP containing puncta since previous studies have shown that FMRP interacts with translation machinery within these puncta. On Puromycin treatment, we observed a decrease in the size and number of FMRP puncta indicating that these puncta containing actively translating ribosomes. Similar results were obtained with neurons over-expressing dephosphorylated FMRP (FMRP S500A).

Our next goal was to investigate the domains of FMRP necessary for microtubule association. We performed a microtubule enrichment assay with HEK293T cells transfected with full length and domains of FMRP. Through the stabilization of microtubules using Taxol, we observed that none of the individual domains of FMRP were enriched in microtubule pellet as efficiently as the full-length protein. However fusing the KH domain to either the N-term or the C-term enhanced their enrichment in the microtubule pellet implying that synergistic combination of domains enhances microtubule binding.

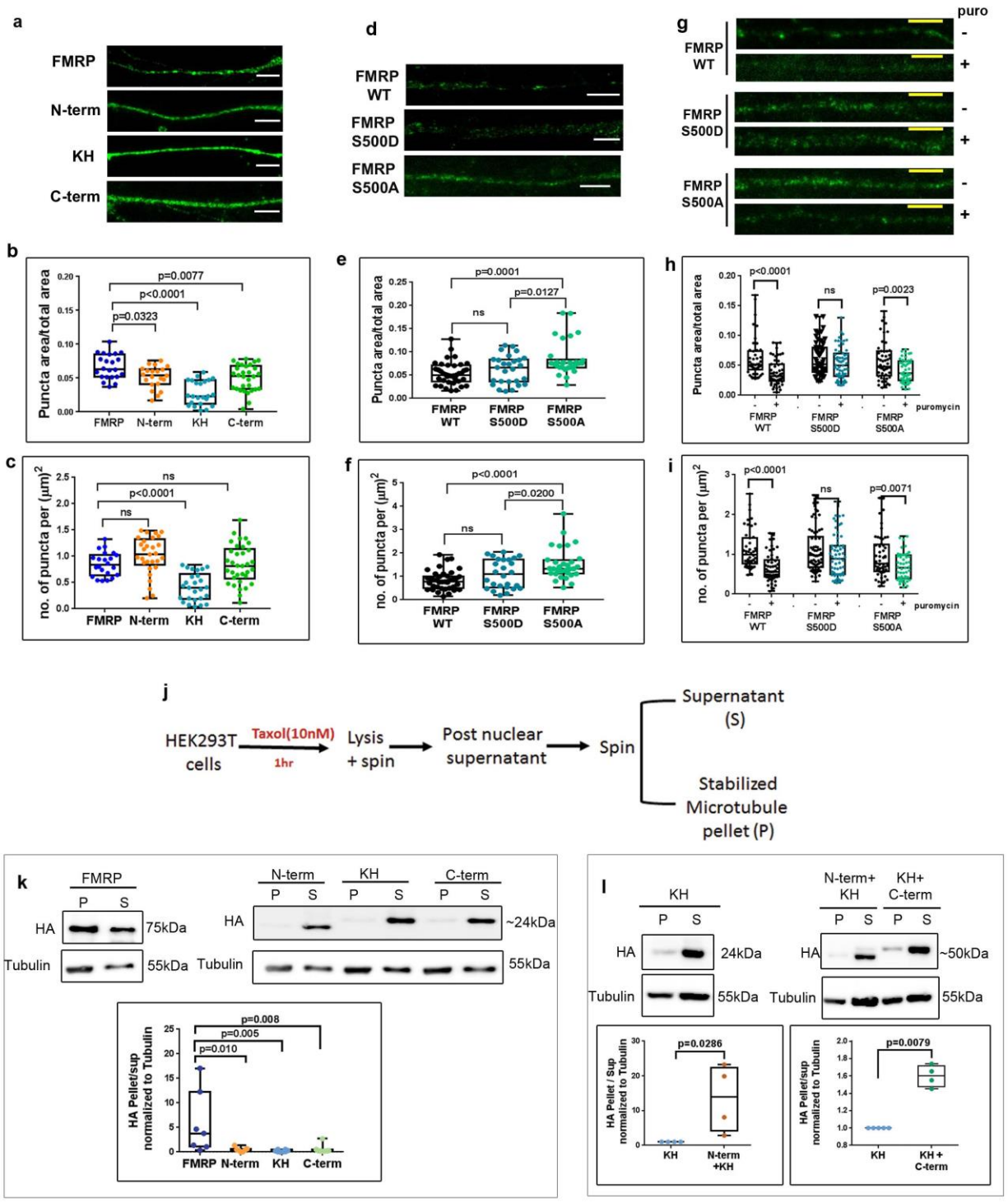

Figure 2: KH domains of FMRP are essential but not sufficient for efficient puncta formation and microtubule association.

a. *HA fluorescent intensities in primary cortical neurons transfected with full-length FMRP and domains showing dendritic localization of puncta. (Scale bar - 5μm)*

b. *Puncta area by total dendritic area for full-length and domains of FMRP. One way ANOVA p<0.001 with Tukey's multiple comparison's test n = 25-35 neurons from 5 independent experiments*
c. *Number of puncta per unit area of the dendrite for full-length and domains of FMRP. One way ANOVA p<0.001 with Tukey's multiple comparison's test, n = 25-35 neurons from 5 independent experiments*
d. *HA fluorescent intensities in primary cortical neurons transfected with full-length WT, S500D and S500A FMRP variants showing dendritic localization of puncta. (Scale bar - 5µm)*
e. *Puncta area by total dendritic area for full-length WT, S500D and S500A FMRP. One-way ANOVA p=0.0002 with Tukey's multiple comparison test n = 20-40 neurons from 5 independent experiments.*
f. *Number of puncta per unit area of the dendrite for full-length WT, S500D and S500A FMRP. One-way ANOVA p<0.0001 with Tukey's multiple comparisons test. n = 25-40 neurons from 5 independent experiments.*
g. *HA fluorescent intensities in primary cortical neurons transfected with full-length WT, S500D and S500A FMRP followed by treatment with Puromycin (1mM) for 1hr showing dendritic localization of puncta. (Scale bar - 5µm)*
h. *Puncta area by total dendritic area for full-length WT, S500D and S500A FMRP on Puromycin treatment. Unpaired t-test. n = 40-60 neurons from 3 independent experiments.*
i. *Number of puncta per unit area of the dendrite for full-length WT, S500D and S500A FMRP on Puromycin treatment. Unpaired t-test. n = 40-60 neurons from 3 independent experiments.*
j. *Schematic for microtubule enrichment assay on Taxol (10nM) treatment.*
k. *Immunoblots of HEK293T cell microtubule pellet enriched on Taxol treatment after transfection with full-length and FMRP domains. Box plots represent the pellet/ supernatant ratio. One-way ANOVA p=0.0053 followed by Dunnett's multiple comparisons test (n=7-8).*
l. *Immunoblots of HEK293T cell microtubule pellet enriched on Taxol treatment after transfection with KH, Nterm+KH and KH+Cterm. Box plots represent the pellet/ supernatant ratio. Unpaired t-test (n=4-5).*

3.Validating the role of FMRP domains through pathogenic FMRP mutations

A majority of FMRP's functions in the cell have been studied in the context of its absence. However, the precise role of its domains in most cellular functions is yet to be elucidated. We made use of pathogenic mutations that were identified in the coding sequence of FMRP to address the contribution of its domains to processes constituting translation regulation[29–32].

Through polysome profiling, we identified that mutations in the KH domains hamper ribosome binding and invariably interfere with FMRP's ability to inhibit neuronal protein synthesis. Although the frame shift mutation in the C-term disrupted FMRP-polysome association, it resulted in an overall increase in neuronal protein synthesis. This could be attributed to the novel 23 amino acids that get incorporated into the ORF of the FMRP[32]. Lastly, we tested the consequence of these pathogenesis mutations on microtubule association and observed that mutations in the KH and C-term domains cause a disruption in microtubule association.

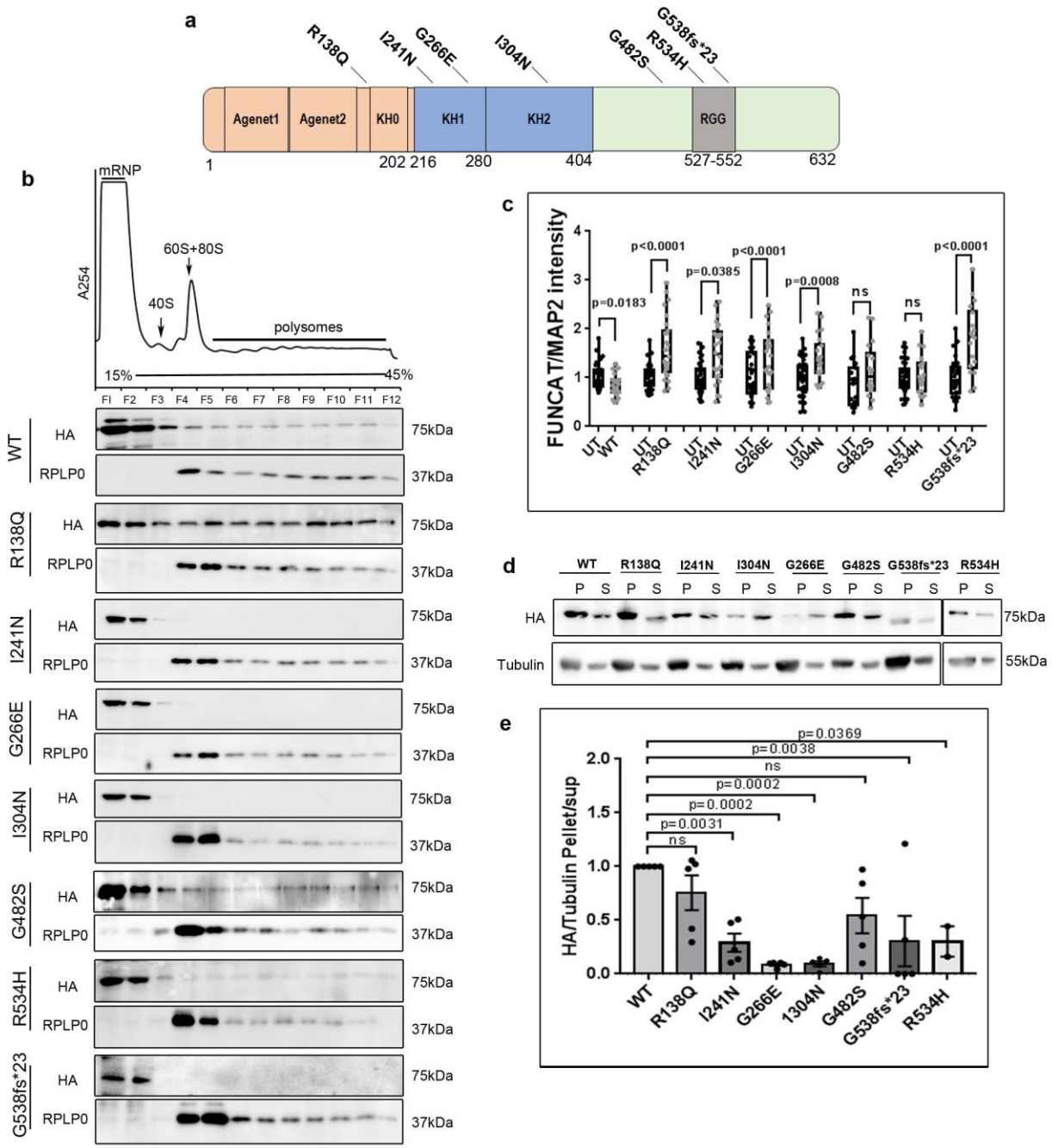

Figure 3: Pathogenic mutations in KH domains drastically alter FMRP's association with ribosomes

a. *Schematic depicting position of mutations in FMRP identified in patients with FXS and intellectual disability.*

b. *Top - Representative polysome trace of HEK293T cell lysate transfected with full-length WT and mutants for 24h. Bottom – Representative immunoblots indicating the distribution of FMRP mutants (probed with HA antibody) with corresponding distribution of ribosome fractions (probed with RPLP0 antibody).*

c. *Box plot representing the quantification of the FUNCAT fluorescent intensity normalized to MAP2 fluorescent intensity for WT and mutants of FMRP. For each FMRP construct, the FUNCAT signal from the transfected neuron was normalized to the untransfected neuron. Unpaired t-test, n= 20-35 neurons from 6 independent experiments.*
d. *Representative immunoblots indicating the enrichment of over-expressed WT FMRP, FMRP mutants and tubulin in microtubule pellet and supernatant on Taxol treatment in HEK293T cells. (n=3-5).*
e. *Bar graph indicating the ratio of enrichment of WT and FMRP mutants in pellet/supernatant on Taxol treatment of HEK293T. One-way ANOVA p=0.0002, followed by Dunnett's multiple comparisons test (n=3-5).*

4.Role of nuclear FMRP in regulating protein synthesis.

Our initial findings were focused on the function of FMRP and its domains in cellular processes that were predominantly cytoplasmic. But there exists a large fraction of FMRP that localizes to the nucleus as well[33,34,36]. Our next goal was to investigate the molecular interactors of FMRP with the nucleus and their implication in translation regulation. For this we examined the distribution of FMRP in the nucleus and in the cytoplasm and estimated around 50% of FMRP to be localised in the nucleus of ESCs. FMRP is also known to interact with a select set of microRNA to regulate protein synthesis[20,42]. To gain insights on the nature of small RNA that interact with FMRP in the nuclear compartment, hESCs were subjected to FMRP immunoprecipitation followed by small RNA sequencing. We discovered that nuclear FMRP interacts with a set of C/D Box snoRNA in human ESCs. We observed that this interaction is only restricted to the nuclear compartment and not to the cytoplasm. C/D Box snoRNAs are small nucleolar RNAs that are responsible for guiding the 2'O Methylation epi-transcriptome on rRNA. C/D Box snoRNAs function in a snoRNP complex consisting of scaffolding proteins and a main methyltransferase protein Fibrillarin. Through co-immunoprecipitations using antibodies against both FMRP and Fibrillarin, we observe that these proteins do not interact with each other. Hence it is possible the FMRP and Fibrillarin form complexes with C/D Box snoRNA are independent of each other. Electro mobility Shift Assays with purified His-FMRP and SNORD80 snoRNA confirmed that the interaction of FMRP with snoRNA is direct. Hence this data summarises that FMRP interacts with snoRNA in the nucleus and this is a novel role of nuclear FMRP to regulate protein synthesis.

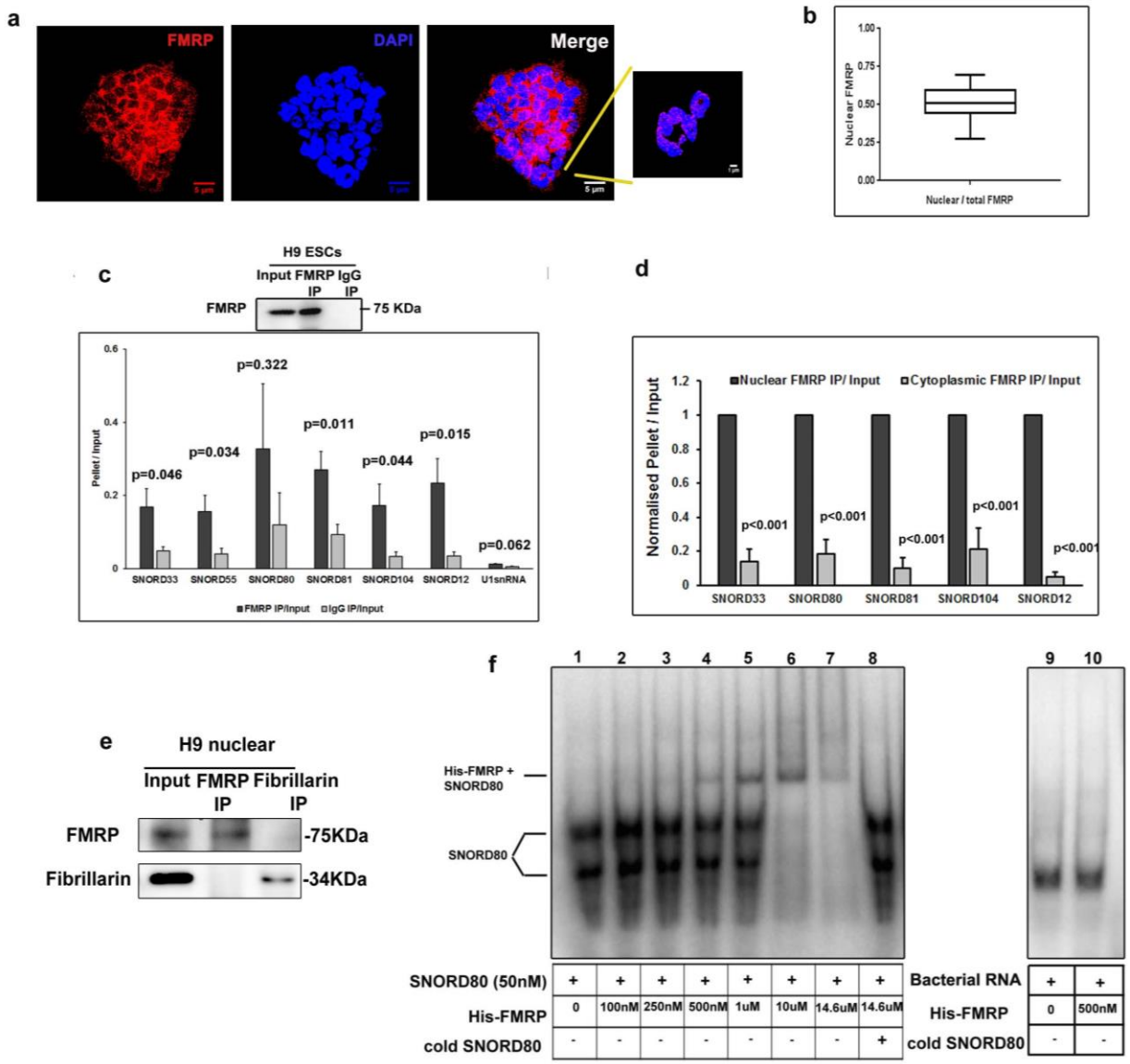

Figure 4: FMRP localizes to the nucleus and interacts directly with snoRNA

a. *Immunostaining of human H9 ESCs (blue-DAPI, red-FMRP, and scale bar, 5 mm) followed by segmented images showing nuclear distribution of FMRP (scale bar, 1 mm).*
b. *Quantification of nuclear FMRP in H9 hESCs, n = 29 cells.*
c. *Validation of FMRP-interacting snoRNA in human H9 hESCs by qPCR with representative immunoblot for FMRP IP (n = 6, unpaired Student's t test, mean+/- SEM).*
d. *qPCR for selected snoRNAs after immunoprecipitation with FMRP from nuclear and cytoplasmic lysates of H9 hESCs. Values are the ratio of pellet/input of the cytoplasmic fraction normalized to the pellet/input ratio of the nuclear fraction (n = 3, unpaired Student's t test, mean+/-SEM).*
e. *Representative immunoblots for FMRP and Fibrillarin followed by FMRP or Fibrillarin immunoprecipitation from nuclear fractions of H9 hESCs. N=3*
f. *Electrophoretic mobility shift assay showing shift in mobility of radiolabeled SNORD80 by increasing concentration of His-FMRP. Lane 8 shows a complete abolishment of shift with*

molar excess of unlabeled (cold) SNORD80 RNA. Lanes 9 and 10 indicated no change in the mobility of His-FMRP with radiolabeled non-specific bacterial RNA. Samples in lanes 9 and 10 were run on a separate gel.

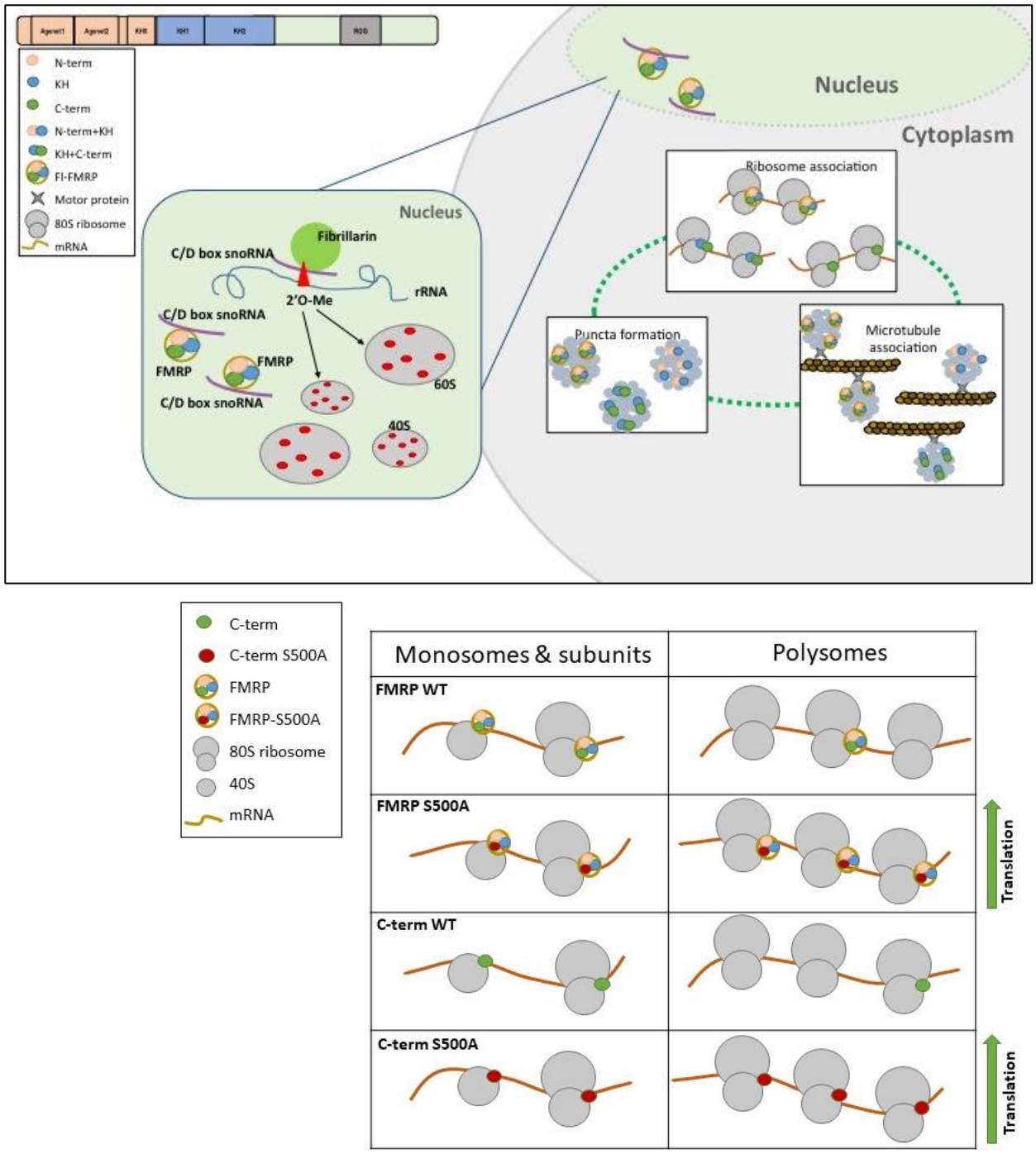

Figure 5: Models summarizing the role of nuclear FMRP and the contribution of individual domains of FMRP to translation regulation.

a. *Minimal domains of FMRP that is required for cytoplasmic processes of puncta formation, microtubule and ribosome binding. In the nucleus, FMRP associates with C/D Box snoRNA to modulate 2'O Methylation on rRNA.*
b. *C-terminus of FMRP is sufficient to regulate the translation function of FMRP in neurons. This function is further regulated through the dephosphosphorylation of FMRP, which increases translation through increased association with ribosomes.*

Concluding remarks:

The primary goal of this project was to investigate the contribution of FMRP to translation regulation through its association with the primary component of translation machinery: the ribosome. FMRP as an RBP has been implicated to regulate the translation of its target mRNAs at various stages of protein synthesis i.e., at translation initiation and at the elongation stage of translation. But the common molecular player among these stages is the ribosome itself. In order to investigate our hypothesis, we used two separate approaches. First, we made use of a structural approach to dissect out the predominant domains of FMRP responsible for ribosome binding and thus in translation regulation. But localization studies indicate FMRP to exist in the cytoplasmic and nuclear compartment as well. Hence as a second approach, we investigated the role of nuclear FMRP and its RNA interactors in regulating protein synthesis.

Through in vitro experiments and polysome profiling we show that the C-terminus of FMRP is sufficient to bind to ribosomes and polysomes akin to the full-length protein. Most importantly it is evident that the C-term domain alone can repress protein synthesis in neurons. Altering its phosphorylation status at Serine 500 further modulates this function of the C-term. Interestingly the KH domains take precedence in the processes of granule formation and microtubule association. It is worth mentioning that although the KH domains were important for these functions, they were not sufficient enough for efficient puncta formation and microtubule binding. We could validate these observations with FMRP containing pathogenic mutations in each of the domains individually suggesting that the domains display a functional hierarchy. This section of our work clearly indicates that FMRP exhibits a ranking in the functionality of its domains in cellular mechanisms leading to translation regulation.

Lastly we examined the role of nuclear FMRP in regulating protein synthesis. Our discovery of the FMRP-snoRNA interactome indicated that FMRP- mediated regulation of translation could begin in the nucleus itself, since snoRNA guide the epi-transcriptome of ribosomal RNA. This distinct complex of FMRP-C/D Box snoRNA was found to be independent of the primary methyltransferase Fibrillarin indicating that FMRP may indirectly regulate rRNA epi-transcriptome.

Future directions

This study focused on the contribution of individual domains of FMRP in its primary role as a regulator of protein synthesis.

1. Our data indicates that the C-terminus is sufficient to mimic the translation inhibition function of full-length FMRP. While we indicate that this function is through a direct interaction with the 80S ribosome, we cannot comment if this interaction is through interaction with mRNA or through

a component of the ribosome itself. Further in vitro binding assays with FMRP and 80S followed by Cryo-EM analysis will confirm the nature of binding in this interaction.
2. We demonstrate the collective role of all domains to enhance the microtubule-binding ability of FMRP. This is based on the enrichment of individual FMRP domains and domain-combinations in the crude microtubule pellet. Hence, we cannot comment on the exact molecular mechanism guiding the interaction of FMRP with cytoskeleton components or regarding the motility of microtubule-bound FMRP complexes. Further, we were unable to capture stark differences in the characteristic of puncta generated by the individual domains alone. Our next goal is to investigate the role of domains in puncta formation and microtubule association in a system devoid of FMRP (FMR1 KO Neurons) so as to avoid the influence of endogenous FMRP.
3. This study also focused on the interaction of FMRP with C/D box snoRNA in the nucleus. It suggests that the interaction of FMRP with specific C/D Box snoRNA might affect 2'O-methylation on rRNA. At the mechanistic level, our data can correlate only the levels of FMRP and the consequent change in methylation of specific sites[9]. We show that FMRP and Fibrillarin (rRNA-specific methyltransferase) are in separate complexes, and we do not know the exact molecular mechanism involving the FMRP-snoRNA complex and how it regulates changes in rRNA post-transcriptional modifications in a cell.
4. We know from our data that FMRP can bind to the ribosome and this could potentially be through the RNA component. Our future goal is to test how the rRNA methylation pattern can be recognized by FMRP through structural-based assays

List of publications

1. Function of FMRP Domains in Regulating Distinct Roles of Neuronal Protein Synthesis. **D'Souza MN**, Ramakrishna S, Radhakrishna BK, Jhaveri V, Ravindran S, Yeramala L, Nair D, Palakodeti D, Muddashetty RS.Mol Neurobiol. 2022 Oct 1. doi: 10.1007/s12035-022-03049-1. Online ahead of print.PMID: 36181660.
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